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Proposed Standard
for
Non-treponemal Tests for Syphilis

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October 1980

U.S. DEPARTMENT OF HEALTH, AND HUMAN SERVICES
PUBLIC HEALTH SERVICE / FOOD AND DRUG ADMINISTRATION / BUREAU OF MEDICAL DEVICES
8757 GEORGIA AVENUE / SILVER SPRING, MARYLAND 20910

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Proposed Standard

for

Non-treponemal Tests for Syphilis

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TABLE OF CONTENTS

	Page Number
Foreword	1
Rationale	3
1.0 Definition of the Device Class	5
2.0 Scope of the Standard	5
3.0 Glossary	7
4.0 Performance Requirements	10
5.0 Quality Assurance and Manufacturing Practices Requirements	17
6.0 Labeling Requirements	18
7.0 Certification	25
8.0 Evaluation Procedures and Requirements	25
9.0 Reference Methods	26
10.0 Reference Materials	27
11.0 Bibliography	27

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Proposed Standard for Non-treponemal Tests for Syphilis

FOREWORD

Under the provisions of the Federal Food, Drug and Cosmetic Act, the Food and Drug Administration is charged with certain responsibilities and regulatory authority to implement programs that will provide reasonable assurance of the safety and effectiveness of medical devices. In partial fulfillment of that charge, the Bureau of Medical Devices maintains a standards program, consisting of:

1. Development of performance standards;
2. Technical support of and participation with voluntary standards organizations;
3. Problem definition studies, for use in standards development; and
4. Publication of standards and reports of studies sponsored by the Bureau.

This publication is a medical device performance standard, developed by the Bureau of Medical Devices, Division of in vitro Diagnostic Device Standards, in cooperation with the Center for Disease Control (CDC).

For a number of years, the Center for Disease Control has operated a program of voluntary premarket review and batch certification for products used in the diagnosis of veneral diseases. This program has wide acceptance in the medical community and has been endorsed by many State Public Health Departments as a requirement for tests used in socio-legal screening programs. This standard is devised to codify requirements for the various stages of product development and manufacturing which, based on CDC experience, must be controlled by the manufacturer to ensure efficacy of these devices.

At the time of publication, this standard is not being promulgated under the provisions of Section 514 of the Federal Food, Drug and Cosmetic Act. Compliance, therefore, is not required by Federal law. Conformance to the requirements in this standard is voluntary. However, readers and potential users of this standard should recognize that compliance may become mandatory under certain circumstances, for example:

1. When referenced in Federal regulations;
2. When referenced in regulations promulgated by State and local regulatory agencies; and
3. When referenced in procurement specifications.

This standard is subject to periodic review and revision. Individuals and organizations desiring to make comments or suggestions concerning this standard are invited to submit them to:

Division of in vitro Device Standards (HFK-320)
Bureau of Medical Devices
8757 Georgia Avenue
Silver Spring, Maryland 20910

RATIONALE

In reviewing the history of non-treponemal tests for syphilis, it became apparent that there are three circumstances in which problems can arise:

1. Deficiencies in field trials during the developmental stages result in inaccurate estimates of performance characteristics;

2. Breakdown in manufacturing controls result in production batches which fail to perform as the material used to develop performance claims did; and

3. Manufacturers of products which may be expected to be identical, e.g., VDRL Reagents, establish slightly different release criteria, resulting in between manufacturer bias of results.

For a number of years the Center for Disease Control, Bureau of Laboratories, has addressed these problems through a manufacturers assistance program. The developmental aspects of new tests have been dealt with by establishing a committee whose members interact with the individual manufacturer, advising on and supervising field trials, performing in-house evaluations of pilot batches of product, and involving themselves in the project in whatever way seems necessary and appropriate. It is a flexible approach and the extent of CDC involvement has varied from product to product.

After the developmental review is complete, the Biologic Products Division, Diagnostic Products Evaluation Branch works with the manufacturer to develop a testing procedure and criteria which will be used and published as CDC specifications for the product.

When non-treponemal tests for syphilis were first identified as high priority devices for standards development by the FDA Diagnostic Products Advisory Committee, there was a presumption that the CDC specifications could be adopted as standards with only minor modifications. It subsequently became apparent that the specifications were, in fact, only the end product of a much more involved premarket review process. In order to assure the efficacy of these products it is necessary for the standard to address the entire process.

This change in approach was reviewed by the Microbiology Subcommittee of the FDA Diagnostic Products Advisory Committee and then implemented through a cooperative effort of CDC and FDA staff. Responses received by FDA after requesting information to develop a standard (FR 40:17058-17059) were also reviewed as part of the development process.

The section on performance limits addresses the design of field trials for developing performance claims by describing the types of populations which should be included and, by specifying confidence limits, providing some guidance as to the size of the population. Limits of acceptable performance are established in relation to certain population groups. For other population groups no limits are set, either because the nonspecific nature of the test makes limits difficult to judge or because of low incidence of the category. The manufacturer is required to provide information in labeling regarding such groups.

When performance limits are set they have been based on observed performance of currently marketed products in studies recently reviewed by CDC.

Batch performance is addressed by providing interpretive guidelines related to the "Device Current Good Manufacturing Practices" Regulations (21 CFR 820) related to known characteristics of the products. The recommendation of an Acceptance Quality Level (AQL) as a guide for the design of Quality Assurance systems is adopted from Department of Defense (DOD) procurement procedures. The specified AQL is that which is commonly used in DOD procurement specifications for these and other in vitro diagnostic devices.

Similarly, interpretive guidelines for "Labeling for in vitro Diagnostic Products" regulations (21 CFR 809.10) are provided which relate to known characteristics of the products. CDC formula specifications for certain products, which are presently incorporated into some state regulations, have been addressed by providing an official name-formula relationship in the labeling interpretations.

The section on certification provides an incorporation of the CDC voluntary program for premarket evaluation of each production batch of these products. Only firms participating in that program can be considered as completely in conformance with this standard.

Proposed Standard
for Non-Treponemal Tests for Syphilis

1.0 Definition of the Device Class

This standard establishes criteria for the performance of devices intended for use in the diagnosis of syphilis or as diagnostic aids in the identification of syphilis which do not specifically identify Treponema pallidum or specific antibodies to T. pallidum. Currently marketed products in the class use a cardiolipin-type antigen to detect reagin in serum or plasma, but the class is not to be specifically limited to these methodologies. Criteria for products or methods to detect neurosyphilis by the detection of reagin in cerebrospinal fluid are not included in this document.

2.0 Scope of the Standard

Non-treponemal tests for syphilis are widely used for a variety of reasons and circumstances. The following examples illustrate some of the most frequent uses:

(1) Screening (initial testing) large numbers of individuals in low-incidence populations to detect the occasional asymptomatic patient (e.g., school or employment physicals, any complete physical examination);

(2) Screening individuals in circumstances where health hazards may be considered to be higher than under ordinary circumstance (e.g., premarital, prenatal, hospital patients' admission routines);

(3) As a preliminary indicator in the diagnosis of high incidence, high risk, or symptomatic patients in cases where treatment should not be delayed while a treponemal confirmatory test is sent away to a reference laboratory; and

(4) As a quantitative method to follow the course of the disease and monitor the response to treatment.

Tests performed for any of these purposes may be performed in any type or size of laboratory, covering the full range from the doctor's office to the central laboratory of a major medical center. The same products may be used regardless of the level of laboratory sophistication.

All current methodology is based on the reaction between an incompletely defined antigen prepared from cardiolipin, cholesterol, and lecithin and an antibody-like substance called reagin, which has not been completely characterized, either chemically or immunologically. The reaction may be detected by flocculation of a suspension of the antigen, or the antigen may be bonded onto or mixed

with inert particles for easier visibility. One system utilizing charcoal particles also has a variation for an automated system. Other products may detect the reaction by complement fixation methods.

Regardless of the detection system, basic problems are inherent in the nonspecific nature of the test. Traditionally, the specificity and sensitivity of a test or product in this device class have been defined on the basis of large scale field studies, correlating test results with physicians' clinical judgment. This standard codifies the traditional approach, establishing requirements for the design of such studies and setting performance limits in terms of correlation with reference methods as well as with physicians' judgment.

The manufacturers of a product marketed for the diagnosis of syphilis
by means of a non-treponemal method must have data showing that any such product has specificity and sensitivity established by studies of the required design and that the correlation with the reference methods is within the required limits when used as intended.

The manufacturer is also responsible for establishing quality assurance procedures adequate to assure that his product meets the requirements of this standard as well as all other claims which he makes in the labeling of his product and to assure that it will continue to meet them for the duration of the shelf-life of the product. However, in recognition of the lack of chemical or immunological definitions as well as the non-specific nature of the test systems, this standard sets forth requirements which must be included in any manufacturer's quality assurance plan.

In addition to the manufacturer's internal controls, the nature and use of these products requires, for a given methodology, e.g., VDRL, which may be produced by any number of manufacturers, that there be some uniformity in the adjustment of reactivity from manufacturer-to-manufacturer. In order to assure the reliability of results from this aspect, this standard establishes a requirement for batch certification of these products, as conducted through the voluntary pre-market evaluation program, Diagnostic Product Evaluation Branch, Bureau of Laboratories, Center for Disease Control.

3.0 Glossary:

This glossary contains a list of definitions of terms encountered in the Proposed Standard for Non-Treponemal Tests for Syphilis with which the reader may not be familiar or for which a definition is given that specifically applies to this Standard. Where possible terminology or definitions recommended by the World Health Organization or given consensus recognition through the National Committee on Clinical Laboratory Standards have been used.

Acceptance Quality Level (AQL): The AQL is the maximum percent defective (or the maximum number of defects per hundred units) that, for purposes of sampling inspection, can be considered acceptable as a process average [Mil Std 105-D Military Standard Sampling Procedures and Tables for Inspection by Attributes].

Acute False Positive Reaction: Repeatedly reactive results with non-treponemal tests, not confirmed by treponemal tests or clinical evidence, persisting six months or less.

Antigen: The substance in the non-treponemal test system which is capable of reacting with reagin or antitreponemal antibody, usually cardiolipin-lecithin compounds.

Cardiolipin: A substance derived from beef heart, probably a component of myocardial membranes.

Certification: Testing of samples from each batch to verify that the level of reactivity is within the acceptable range for that methodology; in this instance, evaluation by the Diagnostic Product Evaluation Branch, Division of Biologic Products, Bureau of Laboratories, CDC to establish that CDC specifications are met.

Chronic False Positive Reaction: Repeatedly reactive results with nontreponemal tests, not confirmed by treponemal tests or clinical evidence, persisting longer than six months.

Confidence Limits: The limits of an interval within which it can be stated -- with a specified level of confidence -- that an estimated parameter lies.

Conformance Test Procedure: A procedure used for determining conformance of a device to the requirements of a standard.

Interference: The deviation in test result caused by a substance or a condition other than treponemal infection.

Interference False Positives: Chronic or acute false positive reactions from an individual known to have a substance or condition which has been identified as interfering with the non-treponemal test.

Non-Treponemal Reference Method: The non-specific method used on a defined population to provide results with which the device (Test Method) results will be appropriately compared during the developmental testing and/or the conformance test procedure.

Non-Treponemal Test for Syphilis: Tests used in the diagnosis of syphilis or as diagnostic aids in the identification of syphilis which do not specifically identify Treponema pallidum or specific antibodies to T. pallidum.

Normal False Positives: Chronic or acute false positive reactions from an apparently healthy individual in whom the presence of interfering substances or conditions cannot be established [previous terminology Biological False Positive].

Qualitative Test: A non-treponemal test for syphilis designed to demonstrate the presence or absence of syphilis-associated reagin or antibody in a specimen.

Quantitative Test: A non-treponemal test for syphilis designed to demonstrate the amount of syphilis-associated reagin or antibody present in a specimen.

Reactive: A specimen which contains reagin or antitreponemal antibody capable of interacting with the antigen in a non-treponemal test system; term used to report a positive qualitative test.

Reactivity: The reported result of a qualitative test, e.g., Reactive, Weakly Reactive, Non-Reactive; a descriptive term for the relative strength of the visual indicator system, e.g, size clumps in VDRL system.

Reagin: Antibody or antibody-like substances capable of reacting with cardiolipin antigen in a non-treponemal test system.

Reproducibility: Estimate of precision usually defined by round-robin testing of a panel of blinded replicate specimens to determine the percentage agreement of qualitative results and/or quantitative units between-technicians-within-laboratory, within-day and between-day-within-laboratory, and between laboratories.

Screening Test: A non-treponemal test intended for use in populations with a very low incidence of syphilis which may give a somewhat higher rate of false positives as a trade-off for a decreased false negative rate.

Sensitivity: The rate of false negatives reported by a non-treponemal test system, stated as a proportion or percentage calculated by dividing the number of false negatives by the number of individuals tested who are identified by other means as having active syphilis or residual anti-treponemal antibodies from previous infection.

Specificity: The rate of false positives reported by a non-treponemal test system, stated as a proportion or percentage calculated by dividing the number of false positives by the number of individuals tested identified by other means as having neither active syphilis nor residual treponemal antibody from previous infection.

Specimen: Material removed from the human body for the purpose of diagnostic testing; within the scope of this document, serum or plasma as specified in directions for use of the device.

System: A combination of components arranged for the purpose of providing an analytical or diagnostic result.

Test Method: For non-treponemal tests for syphilis, that procedure which incorporates the device to which this standard is applicable.

Titer: In a quantitative immunological test, the highest dilution showing a positive (reactive) result, expressed as the ratio of the dilution to its reciprocal.

Treponemal Reference Method: The method used, in conjunction with clinical diagnosis, to confirm the disease-state classification of an individual in the test population e.g., Normal, Non-Syphilitic, Syphilitic, Previous infection, Residual Anti-Treponemal Antibody; The Fluorescent Treponemal Antibody-Absorbed Test (FTA-ABS).

Treponemal Test: Tests used to confirm the diagnosis of syphilis which employ an antigen prepared from Treponemal organisms to react with antibodies which are specific to T. pallidum.

4.0 Performance Requirements

The critical characteristics of products intended for use in the detection of syphilis by non-treponemal methods are sensitivity and specificity as determined for a particular population group. Requirements are therefore established to assure the validity of information regarding sensitivity and specificity, and limits are established for the specificity and sensitivity as defined for designated population groups.

4.1 Characterization of Method and System

4.1.1 Performance characteristics of the product when used for the defined population groups (see Table 1) shall be established within the confidence limits stated for that group. Data and analyses to support performance characteristics and confidence limits must be available on request.

4.1.2 When minimum limit is stated, performance characteristics must meet or exceed that limit. When limit is stated as acceptable range, performance characteristics may not fall outside that range. If limit or range is not stated specifically, manufacturer must provide information regarding that performance characteristic established within the designated confidence limit or stating the confidence limit within which the information is established if no confidence limit is specified for that group.

4.1.3 Any unique or unusual characteristic which may be claimed to distinguish the product from others in the product class must be described and validated in a manner similar to the common characteristics, including a statement of confidence limits.

4.1.4 Intended use will be considered and performance limits may be set for products with limited intended use, i.e., screening tests. In cases where a product or method is generally or historically recognized to have additional uses, the product must meet requirements for all generally recognized uses.

Alternatively, a product marketed for a limited use, under specific performance requirements, may be labeled clearly to state the intended use, limitations, including the performance characteristics which render this particular product unsuitable for other recognized uses, must be fully explained in labeling.

4.1.5 In establishing supporting data for any of the defined population groups [4.1.6 and Table 1] the manufacturer shall maintain records of any specimens which are eliminated from the final analysis. Such records shall include an explanation of the rationale for such elimination, and shall be available on request.

4.1.6 Requirements for particular populations groups are as follows:

4.1.6.1 Normal individuals, clinically negative for any disease, sera nonreactive by both VDRL and FTA-ABS:

The proportion of false positives shall not exceed 2.0% for general qualitative products or 7.0% for products intended for use under the definition of "screening test" (see glossary) when calculated by dividing the number of positive test results by the total number of individuals in this category who were tested. The total number of individuals tested in this category shall be sufficient to establish with 95% confidence that the true proportion of false positive is no greater that the stated limit (see Table 1, Category 1a).

4.1.6.2 Normal individuals, clinically negative for any disease, VDRL Reactive, FTA-ABS Nonreactive (Normal False Positive):

The proportion of agreement shall be calculated by dividing the number of positive test results by the total number of individuals in this category tested. No limit is set, but the information obtained regarding this population must be provided in the product labeling. The total number of individuals tested in this category shall be sufficient to establish with 95% confidence that the true proportion is within ±0.1 of the stated proportion (see Table 1, Category 1b).

4.1.6.3 Individuals with non-syphilitic diseases or conditions which have been reported in the literature to cause occasional Reactive VDRL, FTA-ABS Nonreactive (Interference False Positive):

Selection shall be from the following interfering diseases or conditions:

(1) Diseases or conditions reported to cause acute false positive reaction:

(a) Pneumonia, bacterial, or viral;

(b) Hepatitis;

(c) Infectious mononucleosis;

(d) Lymphogranuloma venerium; and

(e) Smallpox vaccinations or other immunization.

(2) Diseases or conditions reported to cause chronic false positive reaction:

(a) Leprosy;

(b) Collagen diseases, eg. systemic lupus erythematosus;

(c) Arthritic diseases or other inflammatory diseases;

(d) Drug abuse, current or recent (1 yr) history; and

(e) Aging.

A minimum of 5 diseases or conditions shall be included and no single disease or condition shall make up more than 20% of the total population. The proportion of false positives with the VDRL and with the test shall be calculated for each disease separately and for the population as a whole. For general tests the total proportion of false positives for the test shall not exceed 5% or shall not exceed the proportion of VDRL false positives by a ratio of greater than 1.25, whichever is greater. For products meeting the provisions for screening tests the limits shall be 10% or a ratio not to exceed 1.5, whichever is greater.

The respective proportions for each individual disease or conditions shall be included in the labeling. Any individual disease or condition which is found to have a test false positive rate to exceed 25% may be excluded from the calculation of the total proportions, providing that product labeling clearly indicates that the product is unsuitable for use in patients with such disease or condition (see Table I, Category 1c).

4.1.6.4 Individuals with Syphilis at the time of testing having:

(1) Primary Syphilitic lesions, Darkfield +, VDRL Nonreactive, FTA-ABS Nonreactive: The proportion of test positives in this category, if any, shall be calculated and included in the labeling (see Table I, Category 2a).

(2) Presumptive Primary Syphilitic lesions, Dark-field +, VDRL Nonreactive, FTA-ABS Reactive: The proportion of test positives in this category, if any, shall be calculated and included in the labeling (see Table I, Category 2b).

(3) Presumptive Primary Syphilitic lesions, Dark-field +, VDRL Reactive, FTA-ABS Reactive: The proportion of test positives in this category shall be calculated and included in the labeling (see Table I, Category 2b).

From the analyses of the three subcategories of individuals with Primary Syphilis the manufacturer shall estimate:

(1) The time of occurrence of seroconversion in relation to the FTA-ABS;

(2) The time of occurrence of seroconversion in relation to the VDRL; and

(3) The sensitivity (% of positive test results) for each subcategory and for the total Primary Syphilis population tested.

The total number of individuals tested in these categories shall be sufficient to establish with 95% confidence that the true proportion is within +0.05 of the stated proportion for the total Primary Syphilis estimate of sensitivity.

4.1.6.5 Individuals with current diagnosis or history of active syphilis, no primary lesions, no interfering disease, untreated at the time tested, VDRL Reactive, FTA-ABS Reactive: The proportion of positives for tests (general or screening) shall not be less than 98%. The total number of individuals tested in this category shall be sufficient to establish with 95% confidence that the true proportion of the false negative does not exceed 2.0% (See Table 1, Category 2d).

4.1.6.6 Individuals with a history of Syphilis, treated over one year previously, no interfering disease, VDRL Nonreactive, FTA-ABS Reactive: The proportion of reactive results shall be calculated and included in the labeling. Analysis of this data shall be used to estimate the probability that the product will erroneously identify adequately treated syphilis as latent.

4.1.6.7 Individual with a clinical diagnosis of late syphilis, VDRL Reactive or Non-Reactive, FTA-ABS Reactive: The proportion of reactive individuals in this category shall be calculated for both the test and the VDRL and shall be included in the labeling for comparison. In recognition of the limited incidence of late syphilis, no requirement is set for the confidence limit of this data. The manufacturer shall define the confidence of the data and include the definition as part of the comparison in the labeling.

4.1.6.8 Individuals with no history or evidence of active Syphilis, (latent), VDRL Reactive or Non-Reactive, FTA-ABS Reactive: The proportion of reactive individuals in this category shall be calculated for both the test and the VDRL and shall be included in the labeling for comparison. In recognition of the limited incidence of latent syphilis, no requirement is set for the confidence limit of this data. The manufacturer shall define the confidence of the data and include that definition as part of the comparison in the labeling.

4.1.6.9 Individuals having syphilis at any stage, untreated when tested at the initial identification of the infection (may include either primary or recurrent infection) VDRL Reactive, FTA-ABS Reactive: For products claiming use as a quantitative method, titer for each individual shall be determined by the VDRL and by the test method. Analyses of the matched pairs of results should establish the correlation between the quantitative results of the two methods. The results of these analyses shall be included in the labeling for the product. Since this population group may be expected to include all individuals identified in above categories as VDRL Reactive (Syphilitic), in establishing the quantitative correlation the manufacturer shall analyse the data for each of these population groups separately, to determine whether any bias or other anomaly is associated with any defined population group. The results of such analyses shall be provided in the labeling of the product. For any claim made as a result of the analyses of quantitative test results, the manufacturer shall calculate the range of the 95% confidence limit for the claim and include this information in the labeling (see Table I, Category 4a).

4.1.6.10 Individuals having syphilis at any stage, followed throughout course of treatment: For any product claiming usefulness in following treatment and determining adequacy of treatment, a minimum of 10 individuals shall be followed for at least one year using both the VDRL and the test product to establish antibody response curves. Response curves should include at least 6 points. Correlation of paired results on each specimen in the curve should relate to the claim for quantitation correlation, [see 4.1.6.9 above], or the data shall be considered invalid. Analyses of data shall be by graphic display of paired curves on individual patients followed. Labeling shall include summaries of this study and at least samples of typical paired curves.

4.1.7 All raw data, analyses, etc., from the above studies will be permanently retained by the manufacturer. This information will be available to the Agency for review on request.

Table 1.

	Population Definition	Performance Characteristics	Intended Use	Performance Limit	Confidence Limit
1a.	Normal (Clinically negative for any Disease, VDRL NR, FTA-ABS NR)	p = Proportion False Positive $\hat{p} = x/n$ = Sample Estimate of p x = False Positive n = Sample Size	General (Qual.)	$p \leq 0.02$	95%
			Screening (Qual.)	$p \leq 0.07$	95%
b.	Normal False Positive (Clinically Neg. for all Disease, VDRL R, FTA-ABS NR)	p = Proportion False Positive $\hat{p} = x/n$	Any Qualitative		
c.	Interference False Positive FTA-ABS NR, Disease(s) or Conditions (Non-Syphilitic). Reported to Cause Occasional VDRL R	p_v = Prop. VDRL False Positive p_t = Prop. Test False Positive $\hat{p}_v = x_v/n$ x_v = VDRL False Pos. $\hat{p}_t = x_t/n$ x_t = Test False Pos.	General	$p_t \leq 1.25p_v$ or $p_t \leq 0.05$	
			Screening	$p_t \leq 1.5p_v$ or $p_t \leq 0.10$	
2a.	Syphilis (Untreated) Primary Syphilitic Lesions, Darkfield, VDRL NR, FTA-ABS NR.	p_1 = Proportion Positive Period 1 $\hat{p}_1 = x_1/n_1$	Any Qualitative		
b.	Darkfield ±, VDRL NR, FTA-ABS R	p_2 = Proportion Positive Period 2 $\hat{p}_2 = x_2/n_2$	Any Qualitative		
c.	Darkfield ±, VDRL R, FTA-ABS R	p_3 = Proportion Positive Period 3 $\hat{p}_3 = x_3/n_3$	Any Qualitative		
2a-c	Total Primary Syphilis	$\hat{p}_t = \frac{x_1 + x_2 + x_3}{n_1 + n_2 + n_3}$	Any Qualitative		± 0.05 95%
2d.	Current Diagnosis or History of Active Syphilis, No Primary Lesions, No Interfering Disease, Untreated, VDRL R, FTA-ABS R	p_4 = Proportion Positive Period 4 $\hat{p}_4 = x_4/n_4$	All	$p \geq 0.98$	95%
3a.	Syphilis (Treated) Treatment 1 yr. Previous No Interfering Disease VDRL NR, FTA-ABS R	$\hat{p} = x/n$	Any Qualitative		
b.	Late Syphilis VDRL ±, FTA-ABS R	$\hat{p} = x/n$	Any Qualitative		
c.	No History of Syphilis (Latent) VDRL ±, FTA-ABS R	$\hat{p} = x/n$	Any Qualitative		
	Syphilis at any Stage Initial Identification VDRL R, FTA-ABS R	μ_x = VDRL Titer μ_y = Test Titer	Any Quantitative		
b.	(Small Sample from above Population Followed During Treatment)	μ_x, μ_t as function of time During Treatment	Quantitative with Claims to Monitor Treatment Effectiveness		

5.0 Quality Assurance and Manufacturing Practices Requirements

All products in this product class shall be subject to the general requirements stated in the Current Good Manufacturing Practices for Medical Devices (21 CFR 820). The following specific requirements shall also apply as indicated.

5.1 Products in this class are known to be subject to deterioration as a result of exposure to excessive heat or to bacterial contamination. Therefore, the requirements of §820.46 shall be interpreted to include environmental controls during manufacturing and storage to minimize these potential sources of deterioration.

5.2 Deviations from component specifications, particularly for reagents with official name-formula restrictions, could result in a device in this class being either misbranded or unfit for its intended use. Therefore, the requirements of §820.80(a) shall include provisions for sampling and testing for conformance to specifications.

5.3 The requirement for finished device inspection §820.160 shall be interpreted to include the following characteristics peculiar to the product class:

5.3.1 The active ingredients and principles of reaction of products in this product class are not clearly delineated at the present time. This lack of definitive knowledge, coupled with the nonspecific nature of non-treponemal tests, creates a dependence on final product testing to evaluate lot-to-lot quality. The manufacturer shall determine which physical and chemical properties are critical to the production of lots with uniform performance characteristics and ensure that these properties are monitored [§820.80(a)]. However, batch-to-batch uniformity of these properties cannot be considered to assure product quality, and release of goods shall be based on tests of final product against specimens of known reactivity.

5.3.2 Panels of specimens for testing should be selected to include presumed normal negative, and a range of reactivity for positive reactions, which may include both true positive (FTA-ABS+) and false positive (interference or normal false positive FTA-ABS negative specimens). Specimens should be validated by a treponemal method, and by clinical information where possible. Stability of reactivity of these specimens shall be monitored by use of reference products in parallel with test lots at each use.

5.3.3 Each manufacturer shall be responsible for establishing a sampling plan to ensure the validity of quality assurance testing. Military tables or other statistically-based sampling plans may be used as guidelines, but each manufacturer must consider individual production facilities, lot size, etc., in developing a valid plan. The necessity for destructive testing of products must be assessed against the risk inherent in the release of defective products. Therefore, due to the need to assure homogenity of the batch, and since quality can only be determined by destructive testing, the requirements of §820.160 shall be interpreted as requiring that the total quality assurance program should be designed to provide a minimum "Acceptance Quality Level" of 1.0.

6.0 Labeling Requirements

The general labeling requirements of 21 CFR 809.10(a) and (b) apply to all products included in this product class. The following specific labeling requirement shall also apply as indicated:

6.1 The requirement for proprietary and established name (common or usual name), [§809.10(a)(1) and (b)(1)] shall be restricted as follows:

6.1.1 Specifications and formulation for certain products in this class are associated with generally recognized names as published in recognized or official manuals or formularies. Any product utilizing such a generally accepted name is making a claim regarding the composition and/or performance of that product. The following names may be used only for products with the designated formulations:

6.1.1.1 VDRL Antigen: VDRL antigen for the VDRL Slide Test on serum is a colorless, alcohol solution containing 0.03 percent cardiolipin, 0.9 percent cholesterol, and sufficient lecithin to produce standard reactivity.

Designation and source of components used in production. VDRL antigen should be made from cardiolipin and beef or egg lecithin, prepared as described in the WHO Monograph No. 6, 1955. Before use in compounding antigen, cardiolipin and lecithin should be assayed to determine chemical properties.

These properties should fall within the range of values stipulated in the International Pharmacopeia, Annex to Vol. II. These are:

	Cardiolipin	Lecithin	
		Beef	Egg
Nitrogen	0.1%	1.75-2.0 %	1.75-2%
Phosphorus	4.0-4.30%	3.95-4.20%	3.95-4.20%
Iodoine Number	110-127	70-85	55-60

Chemical methods used for these determinations should be those described in the WHO Cardiolipin Antigen Monograph, 1955.

Cholesterol, C.P., Ash-free (crystallized from alcohol), may be used if odor-free and white in appearance or may be re-crystallized from ethanol or methanol before use.

Ethyl Alcohol, Absolute (A.C.S.)

6.1.1.2 Buffered Saline. VDRL Buffered Saline is used with VDRL Antigen to prepare antigen suspension for VDRL tests for syphilis. It contains the following ingredients:

Formaldehyde Solution (A.C.S.), ml.....0.5
Sodium phosphate, dibasic, anhydrous, (A.C.S.), (Na_2 HPO_4), g.....0.037
Potassium phosphate, Monobasic, (A.C.S), (A.C.S.), Na_2 HPO_4), g.....0.037
Sodium chloride, (A.C.S.), g.....10.0
Distilled Water, ml.....1,000

The pH of the saline is 6.0 + 0.1. Chemicals and distilled water should meet A.C.S. specifications for purity. (Reagent Chemicals, A.C.S. Specifications, 5th. ed., 1975).

6.1.1.3 USR Antigen: USR Antigen Suspension is a stable, ready-to-use reagent for the Unheated Serum Reagin test for syphilis. The reagent consists of centrifuged VDRL Antigen suspension which has been resuspended in a stabilizing solution. (The same antigen suspension is used in the Rapid Plasma Reagin (RPR) and the PlasmaCrit (PCT) tests.)

Designation and source of components used in production:

(1) VDRL Antigen as described above.

(2) VDRL Buffered Saline as described above.

(3) Resuspending Solution prepared from the following:

(a) Phosphate, Thimerosal Solution:

Sodium phosphate, dibasic anhydrous (A.C.S.), Na_2HPO_4), g.....1.42
Potassium phosphate, monobasic (A.C.S.), (KH_2PO_4), g.....1.36
Thimerosal (N.F.), g.....1.00
Distilled water q.s. to 500 ml

The pH of solution is checked to determine that it is in the specified range of 6.9 ± 0.1, and solution is stored at normal room temperature in the dark. May be used for a period of three months.

(b) Choline Chloride Solution:

Choline chloride, g.....40.0
Distilled Water...q.s. to 100ml

Because of its hydroscopic properties, choline chloride should either be dried, as described in USP XIX, page 731, before weighing, or the entire contents of a previously unopened 250 gram bottle of the chemical may be dissolved in distilled water to a final volume of 625 ml. The solution is filtered and stored at normal room temperature. May be used for a period of one year.

(c) EDTA Solution:

(Ethylenedinitrilo) tetraacetic acid, disodium salt (A.C.S),g.....3.72
Distilled water.....q.s. to 100ml

Solution is stored at normal room temperature. May be used for a period of one year.

(d) Distilled water for all solutions should meet A.C.S. specifications (Reagents Chemicals, A.C.S. Specifications, 5th ed., 1975).

(4) To prepared 100 ml of the Resuspending Solution, the following amounts are combined:

EDTA solution	12.5 ml
Choline Chloride Solution	25.0 ml
Phosphate, Thimerosal Soln.	50.0 ml
Distilled Water	12.5 ml

This solution is prepared each time the USR Antigen Suspension is produced and is used to resuspend the centrifuged VDRL antigen suspension particles.

6.1.2 Modifications of these names may be used, but such modifications must be clearly indicated and readily apparent on both immediate container label and other labeling.

6.1.3 Modifications of these specifications may be sed if all labeling clearly indicates a modified product. In addition, labeling shall fully explain the nature and rationale of the modification, including complete details of any changes in performance characteristics from the original.

6.2 The statement of intended use required by 809.10(a)(2) shall be as follows:

6.2.1 For a system, a statement shall appear such as, "For use as an aid in the diagnosis of syphilis." Limitations of intended use may be expressed to reflect the categories of intended use identified under performance limits of systems, e.g., "For use as a screening test to aid in the diagnosis of syphilis."

6.2.2 For a reagent intended for use in a particular non-treponemal method or system for testing for syphilis, a statement shall appear such as, "Reagent for use in the XYZ non-treponemal test for syphilis."

6.2.3 For instruments, specifically intended for use for non-treponemal tests for syphilis, a statement shall appear such as, "For use with non-treponemal syphilis tests by the ABS, DEF or GHI methods."

6.2.4 For instruments which are an integral part of, or exclusively used as a particular non-treponemal test system, a statement shall appear such as, "For use in the ABC non-treponemal test for syphilis".

6.2.5 The exemption of requirement for this statement under 809.10(a)(10) shall apply to containers with a net contents under 3 ml or with less than 4 cm^2 of surface area available for label.

6.3 The statement of ingredients required under 809.10(a)(3) shall be as follows:

6.3.1 May be presented as one of the following alternatives.

6.3.1.1 May be omitted for products with formulation conforming to an official name:

6.3.1.2 For VDRL antigen products or products in which VDRL antigen is a component ingredients may be stated as follows:

Alcohol solution containing 0.03 percent cardiolipin, 0.9 percent cholesterol, and sufficient lecithin to produce standard reactivity.

or

Ingredient	w/v
Cardiolipin	0.03%
Cholesterol	0.9%
Lecithin	0.21+0.01%
Alcohol	To dissolve

6.3.2 For control sera or plasma shall consist of:

6.3.2.1 Biological source, including proportions in case of any mixture, e.g., 50% human, 50% rabbit.

6.3.2.2 Concentration of serum or plasma if other than undiluted.

6.3.2.3 Titer or range, e.g., 1:2, 1:16, 1:8 to 1:32, of reactivity, if appropriate.

6.4 For control materials of human origin, warnings required under §809.10(a)(4) shall include a statement regarding infectious potential for hepatitis, similar to that required by 21 CFR 660.28(b)(15). The exemption of requirement for this warning under §809.10(a)(10) shall apply to containers with a net content under 3 ml or with less than 4 cm^2 of surface area available for label.

6.5 For the requirement under §809.10(a)(8) for complete name and address on immediate label, containers subject to exemptions under §809.10(a)(10) may bear an abbreviation or identifying logo when the required information is of excessive length.

6.6 Statements of intended use in labeling, as required under §809.10(b)(2), shall include the statement required under paragraph 6.2 above, in addition to any other information the manufacturer may desire to include.

6.7 The summary and explanation of the test required under §809.10(b)(3) should include a discussion of the following:

6.7.1 A brief history of the development of cardiolipin tests for syphilis.

6.7.2 A brief history of the development of the product as marketed.

6.7.3 A thorough explanation of the nature of and rationale for any modifications of the product as compared to similar products.

6.7.4 A brief discussion of the general limitations of non-treponemal tests for syphilis, any limitations the product may have in addition to the general limitations, and advantages the product may provide in this regard.

6.7.5 Any special merits or characteristics of this product compared to other products in the class.

6.7.6 Appropriate references to support the information listed above.

6.8 The statement of principles of the procedure required in §809.10(b)(4) may be made in general terms, recognizing that the exact nature of non-treponemal tests for syphilis is not fully defined.

6.9 The required statement of reagent ingredients in §809.10(b)(5)(i) for cardiolipin-lecithin antigens may be in the following format:

Antigen for this test (XYZ) is a colorless, alcoholic solution containing 0.03 percent cardiolopin, 0.9 percent cholesterol, and sufficient purified lecithin to produce standard reactivity. During recent years, this amount of lecithin has been 0.?1 percent ±0.01 percent.

or

Ingredients	w/v
Cardiolipin	0.03%
Cholesterol	0.9%
Lecithin	0.21 ± 0.01%
Alcohol	To dissolve

6.10 Instructions for reconsititution or mixing required under §809.10(b)(5)(iii) may be omitted under that section and included under §809.10(b)(8), Procedure, in those instances where antigens and other reagents must be prepared fresh for immediate use.

6.11 Required instructions for specimen handling under §809.10(b)(7)(iv) should include the following information:

6.11.1 Whether stored specimens may be used in the test system.

6.11.2 Conditions of storage for such specimens.

6.11.3 Maximum length of time specimen may be stored under such conditions.

6.11.4 Use of heated or unheated serum.

6.11.5 Necessary cautions, e.g., use of contaminated or hemolyzed serum.

6.12 Instructions for specimen collection and handling [§809.10(b)(7)] may be omitted in labeling which deals solely with control materials.

6.13 The requirement for details of calibration, [§809.10(b)(8)(v)] is not applicable.

6.14 The required statement of results [§809.10(b)(9)] may be expressed as a discussion of criteria for determining reactivity. Reference to visual comparison with control sera of graded reactivity should be included if appropriate.

6.15 The required information on limitations of the procedure, [§809.10(b)(10)] shall include a general description of the limitations inherent in nontreponemal systems as a group as well as any specific limitations of the product. Any limits on the intended use of the product, e.g., screening, should be discussed under this section. Specific recommendations for follow-up tests should be made.

6.16 The required statement of expected values [§809.10(b)(11)] may be expressed as the incidence of positive results in low risk (incidence) populations and/or the expected incidence of normal false positives. Populations used to establish these incidence rates must be fully described. Populations studied and positive incidence rates for diseases and conditions reported to cause interference with nontreponemal tests for syphilis as required under the section on performance characteristics for methods and/or systems, should be discussed in this section.

6.17 The required statement of specific performance characteristics [§809.10(b)(12)] shall include detailed description and complete discussion of all performance characteristics to be defined by manufacturers in the table of performance limits for systems or methods. A statement of within day and day-to-day reproducibility shall also be included.

7.0 Certification

For purposes of this standard, participation in the CDC voluntary Premarket Evaluation Program shall be considered to fulfill the identified need for certification of uniform levels of reactivity. Distribution of lots not meeting CDC specifications shall be judged to be nonconformance with this standard.

8.0 Evaluation Procedures and Requirements

The following procedures and requirements shall apply to the evaluation of device performance by the Agency.

8.1 All products shall be evaluated using the product as it is marketed. Materials not furnished as part of the product but indicated in the labeling as necessary for proper use of the product shall be used in evaluation of the product. Any such materials may be selected that meet the specifications of the manufacturer. If the labeling indicates that alternative materials are considered acceptable, any such materials may be selected for product testing purposes. Testing for conformance may include testing with any specimen type or matrix for which the product is represented as useful. Any product which fails to conform to any of the performance requirements established in this standard is not in conformance with this standard.

8.2 Testing for conformance may be done at any time during the life expectancy of the product. The product shall be handled as stated in the labeling instructions for storage, reconstitution, mixing and stability. For example, if the labeling states that a reagent product stored at room temperature is stable for one year and after reconstitution and dilution, is stable for one month when stored in a refrigerator, testing for conformance shall be done in accordance with the stated conditions and within that period of time. An expiration date or representation of the period of product stability constitutes a representation that the product meets the requirements of the standard at any time during the period represented. If there is no expiration date or representation of the period of product stability, the product will be deemed to be in violation of the requirement of §809.10(a)(6) and §809.10(b)(5)(iv) that such information appear in the product labeling.

8.3 Specimens used for this evaluation of product performance will be systematically selected to provide a distribution over a population range appropriate to the use of the product being tested. Selection will be from populations to which the test is normally applied or specified as suitable for use by the producer of the product, to include any population groups for which both performance limits and confidence limits are specified in Table I, Characteristic of System/Method. No selection of specimens on the basis of possible interferences will be made, except that individuals with borderline reactions in the Treponemal Reference Method may be excluded.

8.4 Specimens used in product evaluation will consist of individual specimens or samples from stable pools of human origin simulating patient specimens.

8.5 Components of any system will be evaluated as part of the entire system in which the product is recommended for use. Examples of such components are:

8.5.1 Reagents supplied or specified by the manufacturer of the system.

8.5.2 Instruments supplied or specified by the manufacturer of the system.

8.6 No special effort shall be made to control any laboratory conditions (temperature, light, humidity) which might affect the performance of the product unless such conditions are specified in the labeling for the product.

9.0 Reference Methods

9.1 Non-Treponemal Reference Method

VDRL Slide tests are performed in accordance with standard procedures published in the Manual of Tests for Syphilis, Publication Health Service Publication No. 411, 1969 ed. Qualitative tests are performed as specified in "VDRL Slide Qualitative Test on Serum," pp. 37-38. Quantitative determinations are performed according to "VDRL Slide Quantitative Test on Serum," ppg. 38-39, with the exception that serum dilutions may be prepared in tubes with twofold serial dilutions in volumes of 0.5 ml or more, in order to provide more accurate dilutions for evaluation purposes than the slide dilution procedure described in the cited method.

9.2 Treponemal Reference Method

Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Tests are performed in accordance with standard procedures published in the Manual of Tests for Syphilis, Public Health Service Publication No. 411, 1969 ed., pp. 15-22.

10.0 Reference Materials

The following materials are available to manufacturers and State Public Health Laboratories in limited quantities from the Center for Disease Control:

(1) Reference Antigen for FTA-ABS Test;

(2) Reference Sorbent for FTA-ABS Test;

(3) Reference Anti-Human Globulin, Fluorescein Isothiocyanate (FITC) Labeled, for the FTA-ABS Test;

(4) Reference VDRL Antigen;

(5) Reference VDRL Buffered Saline; and

(6) Reference Syphilitic Sera of graded reactivity.

Requests for information or materials should be directed to:

Biologic Products Division
Bureau of Laboratories
Center for Disease Control
Atlanta, GA 30333

11.0 Bibliography

11.1 Manual of Tests for Syphilis, 1969
Public Health Service Publication No. 411
DHEW PHS, CDC, Atlanta, GA 1969

11.2 Specifications and Evaluation Methods for Immunological and Microbiological Reagents, Vol. 1 - Bacterial, Fungal, Parasitic, 4th Ed., DHEW, PHS, CDC, Atlanta, GA 1975

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